THE AXIAL GRADIENTS IN HYDROZOA.

VI. EMBRYONIC DEVELOPMENT OF HYDROIDS.

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During the summers of 1917 to 1920, spent at the Puget Sound Biological Station, Friday Harbor, Washington, some time was spent in the study of polarity in eggs and embryos of several species of hydrozoa and of modification and control of development through differential susceptibility. Continuation, of the work has not been possible since 1920, but the data already at hand are consistent in themselves and constitute further evidence for the existence and importance in development of the physiological gradients as well as a basis for interpretation in physiological terms of the transformation of the planula into the hydroid form. This opportunity is taken to acknowledge again my obligations to the Director of the Puget Sound Biological Station for the facilities placed at my disposal.

Material and Methods.

Because of its abundance the hydromedusa, *Phialidium gregarium* constituted the chief source of embryonic material. Since the medusæ shed eggs and sperm more or less continuously in small quantities, fertilized eggs were most readily obtained in quantity by keeping medusæ in considerable numbers, often several hundred, in large pans or tubs for a few hours and then collecting eggs and embryos from the bottom. In such material stages may range from newly shed eggs to various cleavage stages or early blastulæ, according to the length of time the medusæ have been in the container. Embryonic stages of *Stomotoca atra* were obtained in the same way, but the medusæ of this species were usually much less abundant than *Phialidium*. Ovarian eggs of all stages of growth were obtained directly from the ovaries of these species. In a third species, *Gonothyræa clarkii*, which was used to some extent, the free swimming medusa stage is absent,

the early embryonic development occurring in gonophores on the hydroid. Planulæ of this species were obtained by keeping the gonophore-bearing hydroids in the laboratory for a few days and earlier stages, by opening the gonophores. The development of *Phialidium* and *Gonothyræa* was followed through the transformation of planula into hydroid stage, that of *Stomotoca* only to the late planula. *Phialidium* and *Gonothyræa* give rise to campanularian hydroids; *Stomotoca* is an anthomedusa.

The axial physiological gradients in eggs and developmental stages were demonstrated as susceptibility gradients by disintegration or cytolysis in KCN in indicated concentrations, *m*/100 to *m*/500 in sea water, $HgCl_2$ in indicated concentrations, *m*/50,000 to *m*/500,000 in sea water, methylene blue and neutral red in low concentrations in sea water; second, as gradients in rate and amount of reduction of $KMnO_4$ in various low concentrations in sea water and in rate of reduction of methylene blue; third, as gradients in rate of penetration of the vital dyes, neutral red and methylene blue. The concentrations of agents used are given as "indicated concentrations" because the normal medium sea water is used as solvent in all cases and the concentration given is merely that indicated by the amount of the agent which is added to a certain volume of sea water. As regards $KMnO_4$ and the vital dyes, the same results are obtained with a wide range of concentrations because these substances are taken up from very low concentrations and accumulate in unchanged or reduced form in the protoplasm. The point of chief importance is that the concentration be low enough so that the staining or disintegration by the dyes and the reduction of $KMnO_4$ shall not occur so rapidly as to obscure the differences at different levels.

A $KMnO_4$ solution, indicated concentration *m*/1,000, served as stock solution and was diluted two to five times or even more for use in demonstrating differences in rate of reduction. Frequently a drop or two of the stock solution was added from time to time as reduction decreased the concentration. Very low concentrations of $KMnO_4$ *e.g.*, below *m*/10,000, produce more or less cytolysis and can be used like other cytolytic agents to demonstrate the gradients. Special attention may be called to the fact that the permanganate gradient is not simply a gradient in the rate of re-

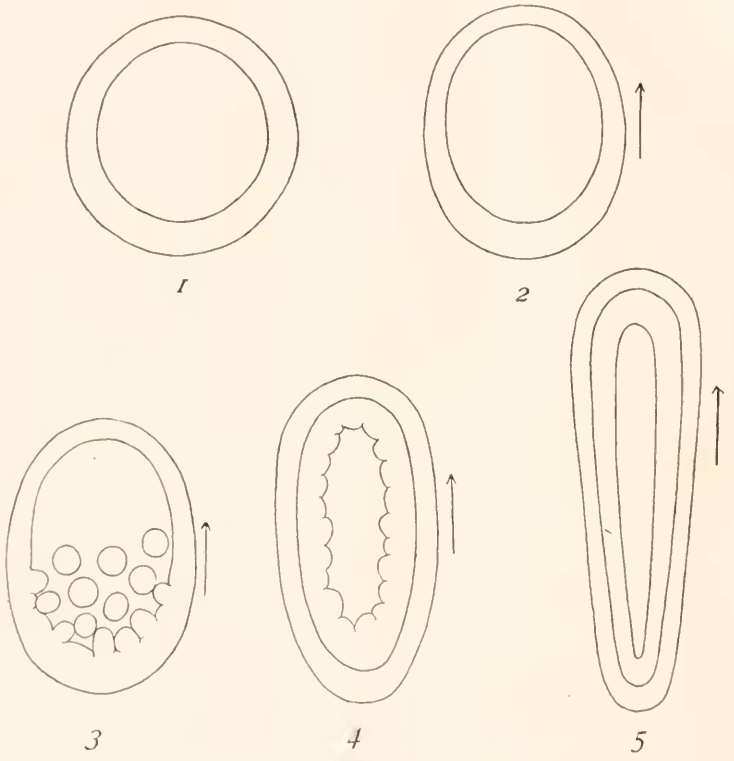
duction of $KMnO_4$ but when the reaction proceeds to completion in excess of $KMnO_4$ a gradient in depth of color indicating differences in amount of reduction is evident. For demonstration of these differences the material is kept in excess of $KMnO_4$ solution 24 to 48 hours and is then dehydrated and cleared since it is opaque in aqueous medium. Such cleared preparations are not permanent in any of the clearing agents thus far used nor in balsam. The brown or blackish color due to MnO_2 or other oxides gradually disappears, apparently by solution, in the course of several days longer. The preparations are sufficiently permanent, however, to permit imbedding and sectioning before the color gradient disappears, though of course fading occurs more rapidly in the sections than in the whole preparations.

For demonstration of the gradients by methylene blue various concentrations, ranging from 1 1,000 to 1 20,000 were used, according to species and result to be attained, the higher concentrations being used chiefly for differential staining, rapid death and disintegration, the lower for demonstration of the gradients by differential reduction and slow disintegration. Concentrations of neutral red used were all very low but were not determined because the results differ only as regards time with all concentrations used and because the dye gradually crystallizes out of solution.

The Polar Gradients During Normal Development.

The course of early development under normal conditions is very similar in the species used as material. Cleavage gives rise to a spherical ciliated blastula (Fig. 1) which soon begins to elongate and shows polarity in the graded thinning of the cell wall decreasing from apical to basal pole, and in locomotion with the apical end in advance (Fig. 2). Immigration of cells from the basal hemisphere, chiefly from regions near the basal pole, begins within a few hours (Fig. 3) and the cells which immigrate apparently undergo further division and arrange themselves as an inner entodermal layer as elongation of the larva continues (Fig. 4). The final form of the swimming planula, attained in *Phialidium* after about forty-eight hours at laboratory temperature, is approximately that shown in Fig. 5, but a considerable amount

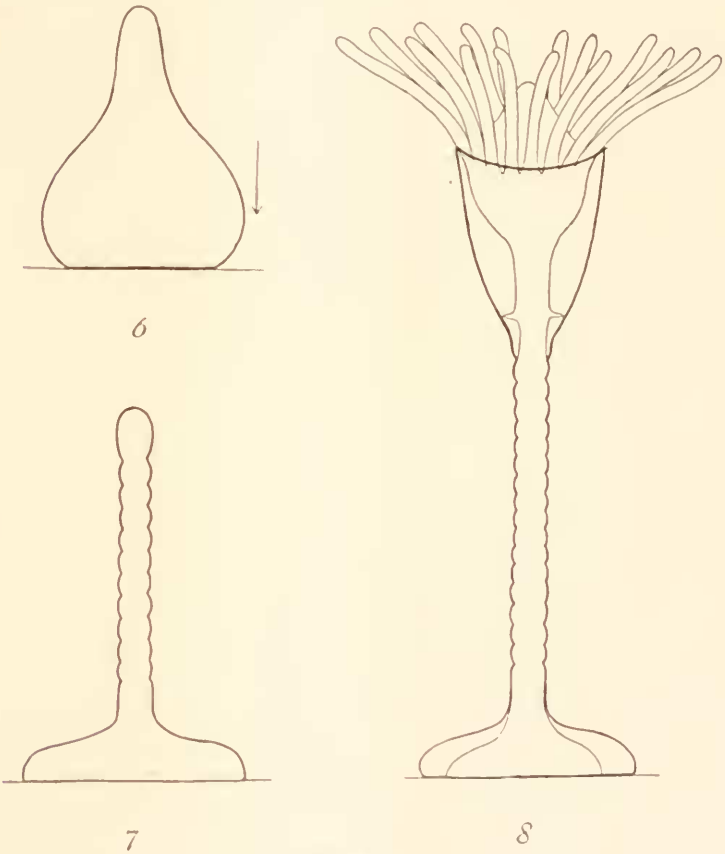
of slow contraction and extension occurs, the same planula being at times almost worm-like in form or contracting to a form considerably shorter and broader than Fig. 5. The original apical end still precedes in locomotion, as indicated by the arrows (Figs. 2–5). After a day or more of continued swimming, either free or along the bottom, the planula attaches itself by the original



FIGS. 1–5.

apical end, which shortens and thickens (Fig. 6), and the basal end begins to elongate to form the stem (Fig. 7) and this in turn gives rise at its tip to the first hydranth (Fig. 8). Stolons may arise later as outgrowths from the attached end. Stem and stolon are easily distinguished since the stem grows erect or free from the substratum and has an annulated perisarc, while the stolon grows in contact with the substratum and is without annulation. This course of development is essentially similar to that described by other investigators for various other hydroid species.

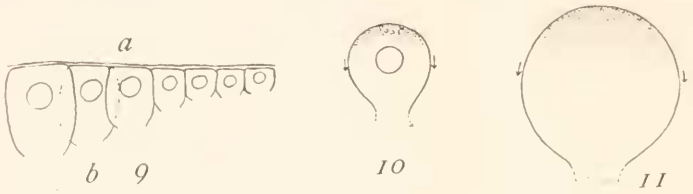
The evidence from the course of development itself, from differential cytolysis, from differential rate and amount of reduction of $KMnO_4$, from differential staining by neutral red and methylene blue and differential reduction of the latter, agrees in indica-



FIGS. 6–8.

ting, first the existence of a physiological gradient along the polar axis from the oöcyte to the planula stage with its high end at the apical end of egg and larva, and second, the appearance in the late planula of a second gradient at the basal end, opposite in direction to the first, and associated with the development of stem and hydranth from this end. Figs. 10 to 17, illustrating diagrammatically the polar gradients of various stages, are in part early stages of the reduction gradients with $KMnO_4$ (Figs. 10–12), in part

early stages of cytolytic gradients. Although complete series of data up to the first hydranth were obtained with cytolysis, with reduction and with differential staining, complete series of figures for the various methods are believed to be quite unnecessary since the results of all agree. The small arrows in Figs. 10–17 indicate the direction in which further progress of reduction or cytolysis occurs. As regards Figs. 10–12 it should also be noted that the shaded portions represent those regions in which the color resulting from reduction of $KMnO_4$ is deepest. All portions of



FIGS. 9–11.

the egg or later stage become diffuse yellow in permanganate within a few moments, but the differential staining of different regions becomes more and more distinct as reduction proceeds, until the increasing opacity obscures the differences and dehydration and clearing are necessary to make them visible.

The growing oöcytes of *Phialidium* form a more or less regular columnar epithelium in the gonad (Fig. 9), each cell being attached by its inner end (*b*) adjoining the radial canal, while the superficial free pole (*a*) is separated from the sea water only by a very delicate membrane covering the gonad. Except in very early oöcytes, the nucleus lies nearer the free pole. Growing oöcytes of various stages, isolated by teasing, rapidly assume rounded form and the nucleus often breaks down or is extruded, but the region of attachment remains distinguishable and serves as a landmark. Such isolated oöcytes show uniformly a gradient in susceptibility with all agents used, in reduction of permanganate and in differential staining, the cytolysis or change in aggregation of the cytoplasm, the reduction and the staining beginning at the free pole and progressing toward the attached pole, as indicated in Figs. 10 and 11. Nucleated and non-nucleated egg fragments also show a gradient which is doubtless identical with that of the

whole oöcyte, though the absence in most cases of any visible basis for orientation of the fragments makes complete demonstration of identity impossible. In the oöcyte of *Stomotoca* the gradient shows the same relation to free and attached poles of the cell as in *Phialidium*.

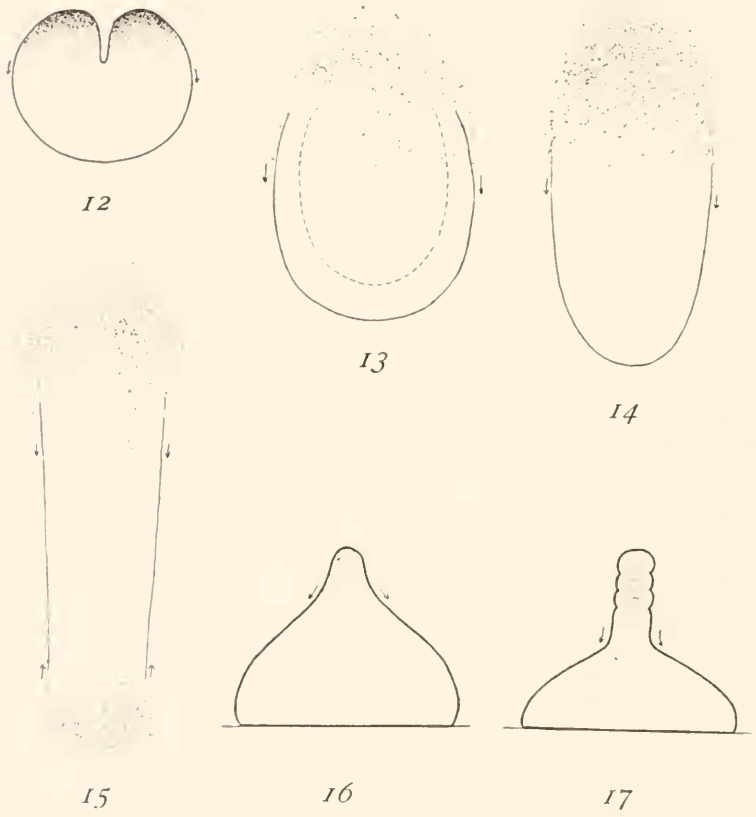
It has been repeatedly pointed out that the various lines of evidence concerning the gradients indicate very clearly that they are associated with quantitative differences in physiological condition involving metabolism as an important factor.[1] In the light of this evidence the inference is justified that the differentials in susceptibility, reduction and differential staining in these hydrozoan oocytes are indicators of a quantitative physiological gradient in the cytoplasm. The relation of this gradient to the free and attached ends of the oöcyte in the gonad suggests that it is determined by the differential exposure of the cell. The intake of oxygen and elimination of Co_2 undoubtedly occur more readily at the free end than elsewhere, and there is every reason to believe that the persistence of such a differential during the growth period will establish a physiological gradient, primarily quantitative in character, involving metabolic and physical factors. In short, the facts at hand indicate, though they do not demonstrate that the physiological polarity of the oöcyte is determined by its differential exposure in the gonad.

The gradient persists after the egg is shed, the polar bodies are formed at its high end, *i.e.*, the pole of highest susceptibility and reducing power, and the first cleavage furrow cuts through the egg from this pole (Fig. 12). During cleavage and early blastula stages a gradient is continuously present, undoubtedly the same as that of the early stages, but absence of definite landmarks makes complete demonstration of identity impossible. As soon as directed locomotion and elongation of the blastula begin, we find that the gradient is apico-basal, the high end being at the apical pole of the blastula (Fig. 13) and the planula (Fig. 14). The facts leave little room for doubt that the gradient, apparently determined by differential exposure in the oöcyte, represents the polar axis of the egg and larva. Moreover, maturation, cleavage,

[1] For discussions of this evidence see Child, '20, '21, Chap. II.; '23b; '24, Chap. VII. and literature cited in these publications.

the differential thinning of the blastula wall and the direction of locomotion of the larva all indicate a greater physiological activity at the apical pole, the high end of the gradient, than elsewhere.

The question whether any gradient exists or arises in the entoderm is of interest, but the only method among those used which



FIGS. 12–17.

is available for demonstrating the presence of such a gradient is that indicating different amounts of reduction of permanganate at different levels. After the entodermal cells have formed a definite internal layer in the planula the cleared permanganate preparations usually show a slight entodermal gradient in amount of reduction, as indicated by depth of color. This gradient is similar in direction to the ectodermal gradient but the differences at differ-

ent levels are less than in the latter. The immigration of the entodermal cells singly and their later arrangement in a layer makes it difficult to believe that this entodermal gradient represents differences present in the cells at the time of, or preceding immigration. If it represents real differences in physiological condition, it is probably superimposed on the entoderm by its relation with the ectoderm, the more active regions of the latter determining, perhaps by transmission, greater activity in the adjoining entoderm.

As the planula elongates a second region of high susceptibility, reducing power and rate of staining gradually appears at the basal end (Fig. 15) and from this region a gradient, opposite in direction to the original gradient, develops. The stage at which this second gradient appears varies somewhat in the different species and in different cultures of the same species. Under good conditions it usually appears somewhat before the planula attains its full length. Under slightly depressing conditions it commonly appears somewhat earlier, but under strongly inhibiting conditions its appearance may be completely inhibited. The portion of the planula body over which the second gradient extends during the free swimming stage varies considerably, being usually one fifth to one third the total length. Undoubtedly the degree of development of this second gradient before attachment depends on the stage at which it first appears and on the condition of the larva.

This second gradient is the first indication of the hydranth-stem axis. As the stage of attachment approaches the activity of the original apical region decreases and the original apico-basal gradient becomes less distinct, while the new gradient at the basal end becomes longer and more distinct. During this period the swimming activity decreases and sooner or later attachment by the original apical end occurs (Fig. 16), a perisarc is secreted and elongation of the basal region to form the hydranth and stem begins (Fig. 17).

Thus far no indications of the original apico-basal gradient have been observed at the stage of Fig. 17. Apparently the only gradient present is the secondary gradient which originated at the original basal end. The objection may be raised that the presence of the perisarc and the attachment of the original apical end

protect this region from the action of external agents. This, however, is not the chief factor in the low susceptibility and reducing power of this region, for when these stages are scraped from the substratum the attached surface is often left exposed, but even then the original apico-basal gradient does not appear. As a matter of fact, various experiments on susceptibility and reducing power of naked protoplasmic surfaces and those covered by perisarc have shown that, except when the perisarc is old and very thick, it is rapidly penetrated by most agents. The very thin perisarc of stages such as Figs. 16 and 17 is penetrated rapidly by $KMnO_4$ and the agents used to determine susceptibility

The tip of the growing stem which represents the high end of the secondary gradient gives rise to the first hydranth (see Fig. 8). This hydranth itself shows a marked apico-basal gradient which is a part of the hydranth-stem gradient. Each tentacle, originating as a localized region of growth on the hydranth body, represents a gradient with the high end at the tip. The tentacle gradient apparently originates in the localization of the region of growth giving rise to the tentacle. This region is not sharply defined, but its activity decreases peripherally from a central area and this decrease evidently constitutes the beginning of the tentacle gradient. The gradients of numerous other axiate organs undoubtedly arise in the same way. The localization of a region of growth giving rise to a tentacle must be determined by correlative factors in the developing hydranth. The tentacles arise at a certain level of the polar gradient and at a certain distance from each other. Each growing tentacle region, once localized, apparently dominates a certain area as does a growing plant bud and determines the course of development of this area. From this viewpoint the tentacle is a gradient in a specialized part which originates at a certain body level in reaction to the polar gradient of the body. In the species serving as material for this paper the tentacles of a circle are apparently not simultaneously determined or localized but the factors concerned in their localization are obscure.

THE PHYSIOLOGICAL SIGNIFICANCE OF THE VARIOUS METHODS.

The first question which arises is whether the axial differences in susceptibility to the various agents used result merely from differences in permeability of the limiting surfaces of the cells. That axial differences in permeability to some, if not to all agents, corresponding more or less closely to the gradients observed, do exist is probable. But that such differences are not the only, nor the fundamental factors in the gradients is indicated by various facts, some of which require brief consideration in connection with the case in hand. First, an observation made repeatedly on oöcytes of both *Phialidium* and *Stomotoca* teased from the gonad is at least suggestive in this connection. As already noted, the oöcytes are attached during the growth period and when they are teased out of the gonad rupture of the cytoplasm occurs at the attached pole and, although the injured region closes within an hour or two, the intact plasma membrane present over other parts of the surface is absent in this region for some time and granules or droplets are often given off into the water. But even while this cytoplasmic region is exposed, it does not become the high end of the susceptibility gradient or the reduction gradient. That is at the opposite pole, in spite of the fact that the agents must penetrate the intact membrane there in order to produce their effects. The only effect of the exposure of the cytoplasm at the attached pole is a little superficial cytolysis or reduction. This absence of relation between the polar gradient of the oöcyte and the injury at the attached pole is striking.

Second, as regards the action of the various agents used to demonstrate the gradients by cytolysis, it is important to note that the cytolytic gradients are the same for agents such as methylene blue and neutral red which penetrate living membranes readily and kill by accumulation within the cells and for those such as $HgCl_2$ which, in the concentrations used, alter and kill the cell surfaces rapidly and so destroy such differences in permeability as may have existed. Again, the cyanides or the CN ion apparently penetrate cells readily and produce their effects largely within the cells, according to recent views by inhibition of the catalyst of oxidation. As a matter of fact, susceptibility to KCN has been shown in various ways to be, within certain

limits a very delicate indicator of physiological condition and particularly of rate of cell respiration. When susceptibility gradients are found to be essentially the same for different agents which act on living protoplasm in ways as different as KCN, $HgCl_2$ and the vital dyes in the concentrations used, it seems impossible that the differences in susceptibility can be due simply to differences in permeability. In this work on the hydroids the use of a large number of agents for lethal susceptibility was regarded as unnecessary for several reasons: first, because differential susceptibility to KCN is believed to be a valuable indication of quantitative differences in physiological condition which in most, if not in all protoplasms, are associated with oxidative processes; second, because the data obtained by different methods, lethal susceptibility, reduction and modification of development, the last to be considered in a later paper, all agree as regards the physiological gradients indicated; and third, because in the light of earlier work on many different forms, with the methods used here and with other methods, the data on hydroid development appear merely as additional evidence in agreement with that already at hand.

And finally, the physiological gradients demonstrated experimentally correspond to differences in rate of the developmental processes. The high ends of the gradients are the most active regions. Differences in permeability are in all probability concerned in the differences in activity, but if this is the case, permeability is merely one factor in the complex of metabolic and other factors which constitute physiological condition.

The basis of the general relation between physiological condition and susceptibility has been discussed elsewhere. At present it need only be pointed out that it does not depend on the particular method of action of a particular agent, but is apparently a relation between a certain degree of disturbance of any kind and the rate of change characteristic of the system disturbed. To a degree of disturbance adequate to bring about disruption or irreversible change of the system in course of time, the more active region is more susceptible because its own activity becomes a factor in producing the total or final effect (Child, '23*b*).

As regards the gradients indicated by reduction of $KMnO_4$, it has already been noted that they appear both as gradients in rate

of reduction indicated by rate of appearance of the brown color and as gradients in amount of reduction indicated by depth of color when the reaction proceeds to completion in excess of the agent and the preparations are dehydrated and cleared. The possibility that a differential permeability along the axis, either of the plasma membrane of naked stages, or of the perisarc in stages possessing it, is a factor in the gradient in rate of reduction need not be denied. It is highly improbable, however, that such differences in permeability are fundamental factors in the gradients of naked stages for $KMnO_4$ in the concentrations used penetrates rapidly and even alters the visible aggregation of the protoplasm before the reduction gradient becomes distinct. Any differences in permeability which may have been present must be destroyed early in its action. Moreover, the possibility that differential permeability determines the gradient in amount of reduction as indicated by depth of color due to oxides precipitated throughout the cell or cells, seems to be eliminated. And finally, the fact that the gradients in rate and in amount of reduction are the same for a particular stage and are also identical with the gradients in susceptibility indicates clearly enough that the different methods of demonstrating the gradients are merely different ways of making evident one aspect or another of the general quantitative gradation in condition of the living protoplasmic system.

That the gradients indicated by reduction of $KMnO_4$ are closely associated with the processes of living in the protoplasm is further shown by the fact that individuals of various stages killed in various ways and then placed in permanganate reduce much less of the agent than living individuals and show, either no traces of axial gradients, or in some cases slight traces for a short time after the action of the killing agent. For example, when developmental stages are placed in alcohol, $HgCl_2$ or various other agents used for histological purposes, then washed and brought into $KMnO_4$ after various periods in the killing agent, it is found that a few seconds in most agents is sufficient to obliterate in large measure the differences on which the reduction gradient depends, though slight traces of a gradient may persist in some cases, even for several hours. In material carried up to 80 per cent. alcohol,

returned to water after a day or two and then brought into permanganate no traces of the reduction gradient have thus far been found. The persistence of traces of the gradients for a short time after action of a fixing agent can scarcely be due to the persistence of a permeability gradient, even if such were present in the living animal. Another interpretation appears more probable, viz., that complete inactivation of the oxidizing enzymes does not always occur at once in the fixing agents, consequently traces of the original differential may persist for a short time. Individuals killed before exposure to permanganate never become opaque black but remain yellow or brownish indefinitely in excess of the agent. Evidently killing before exposure decreases greatly the reducing power of the protoplasm.

The gradients of differential staining with neutral red and the higher concentrations of methylene blue are visible only during the early stages of staining in living material. As the staining of living material progresses the regional differences in depth of color gradually disappear and, so far as the eye can distinguish, the staining becomes uniform throughout long before the depth of color is sufficient to obscure differences. But if staining is continued until cytolysis occurs the cytolytic gradients are the same for any particular developmental stages as those observed with other agents.

The differential staining gradients may represent primarily the differences in permeability of different regions to the dyes, but if appearances can be trusted these differences in permeability disappear as the toxic action of the dyes progresses and in spite of the fact that all regions seem to be stained uniformly, the regions which are more susceptible to other agents and which show greater reducing power are more susceptible to these dyes. Here as in other forms, such differential permeability as may exist appears to be merely one aspect of the differences in physiological condition which constitute the gradients.

In the case of methylene blue the ability of the living protoplasm to reduce and decolorize the dye rather rapidly introduces another factor and makes possible demonstration of the gradients as gradients in rate of reduction and decoloration, particularly in the blastula, planula and later stages, in which the rate of metab-

olism is higher than in early stages. The reduction gradients with methylene blue appear only in relatively low concentrations or after removal to water before staining has progressed too far, but the concentrations and periods of staining suitable for this purpose differ with the different species. For example, the planulæ of *Phialidium* show differential staining with the high regions of the gradient or gradients most deeply stained in the early stages of staining with methylene blue 1/1000, but in 1/5000 scarcely a trace of differential staining occurs, the whole larva staining more or less uniformly. In 1/20,000 there is no appreciable staining during the first two hours of exposure, but after six hours many planulæ show a reversed staining gradient. In the shorter planulæ the depth of staining is least at the apical end of the larva and increases basipetally; in the longer planulæ, in which the second gradient is already present, both ends are slightly or not at all stained and the depth of color increases toward the middle region. Similar reversal of the staining gradient has been observed with low concentrations of methylene blue in various multicellular forms and in ciliate infusoria, including *Paramecium*. These reversed staining gradients in low concentrations result from reduction of the dye to the leuco-compound in the protoplasm. Below a certain concentration, which must be determined experimentally for each species, the higher levels of a gradient reduce the dye as rapidly, or almost as rapidly as it enters and more rapidly than lower levels. Consequently the depth of staining is least at the high end and increases toward the low end of a gradient. Even with concentrations high enough to stain the high regions of the gradients more deeply the reduction gradients often appear when the animals are returned to water before the staining has progressed far. Apparently the reduced dye is toxic, for death and cytolysis finally occur in concentrations so low that the high regions of the gradients which disintegrate first are only slightly or not at all stained. It is improbable that the toxicity of such concentrations, *e.g.*, 1/20,000, can be due to impurities.

Conclusion.

The evidence from the study of the gradients during development agrees with the data of observation in showing, first that

the polarity of the oocyte becomes the polarity of the early planula, and second that a complete reversal of polarity occurs in the course of transformation of planula into hydroid. A simple interpretation of this reversal is possible in terms of physiological gradients. The hydranth-stem axis apparently arises as a bud from the original basal end of the planula. The appearance of the second gradient at the basal end as the planula elongates suggests that the new axis arises through physiological isolation of this basal region from the dominant apical region in consequence of increase in length. Decrease in activity of the apical region, which is indicated by the lower susceptibility of these, as compared with earlier stages of the planula, may also decrease the range of dominance and so play a part in the physiological isolation of the basal region. In these larval stages the range of dominance is undoubtedly very short because the mechanisms of transmission are rudimentary (Child, '15, pp. 149–151) and physiological isolation may occur at a very short distance from the dominant region. In consequence of the isolation the cells at the basal end become more active and lose whatever differentiation they have attained, while the activity of other parts continues to decrease with the progress of differentiation, until the region originally basal and least active becomes the most active region of the body. With further development and with the differentiation of a rudimentary nervous system the range of dominance increases, as in the development of other forms, and the hydranth which arises at the high end of the secondary gradient comes to dominate the whole body, until further increase in length leads sooner or later to physiological isolation again and budding begins. The reversal in direction of polarity in the hydranth-stem axis apparently results from the fact that in the course of elongation of the planula the extreme basal region is the first part to be isolated and to become more active. This precedence makes it the high end of the new gradient. It is evident from the work of many investigators that polarity in the hydroids is extremely labile and readily altered by external conditions. Every hydroid bud represents a new gradient and, so far as can be determined, this bud which forms the first hydranth differs from those which later give rise to branches only in its position at the

opposite end of the polar axis from the original dominant region. In that respect it is similar to the "axial heteromorphoses" which are characteristic features of the normal budding and of the reconstitution of stem pieces in *Tubularia* and many other hydroids (Child, '07*a*, *b* and literature there cited; '15, pp. 91, 92, 133–37). The term "heteromorphosis" is merely descriptive and leaves us without any interpretation of the phenomena concerned. So far as analyzed physiologically, all cases of so-called axial heteromorphosis are essentially cases of budding which involve the origin of a new polar gradient. Actually every hydroid bud and every bud in a multiaxial plant is as truly a heteromorphosis as are these cases of buds arising at the basal end of an axis. All such buds represent the determination of new physiological axes and the conditions, external and internal, determine in each case where the new axes shall appear.

In some of the tubularian hydroids, *e.g.*, *Corymorpha* (Torrey '07, and my own observations), the hydranth-stem axis arises from the original apical end of the planula and although the secondary gradient appears sooner or later at the basal end, physiological isolation is not complete and the secondary gradient gives rise, at least in its earlier stages only to stolon axes, which are inhibited axes (Child, '23*a*). In these forms, then, the primary gradient persists as the gradient of the hydranth-stem axis.

Summary.

1. The axial gradient and its changes during development were demonstrated in *Phialidium* from oöcyte to hydroid stage by differential susceptibility to various agents, differential reduction of $KMnO_4$ and methylene blue and differential vital staining. Developmental stages of *Gonothyræa* and *Stomotoca* served as comparative material. The results of the different methods agree among themselves and with the observed facts of development.

2. In *Phialidium* and *Stomotoca* the polarity of the oöcyte is indicated by a gradient with high end at the free pole, low end at the attached pole. This gradient is directly related as regards direction to the differential exposure of the oöcyte in the gonad and it is suggested that the polarity of the oöcyte is determined by this differential exposure.

3. The high end of the oöcyte gradient becomes the apical pole of the egg at maturation and in early cleavage stages. During later cleavage a gradient is continuously present, but absence of landmarks makes complete demonstration of its identity with the gradient of earlier stages impossible, though there is no reason to doubt that it is the same. From the beginning of locomotion on the identity of the gradient is certain, the high end being the apical, the low end, the basal pole of the larva.

4. As the planula elongates a second gradient opposite in direction to the primary gradient appears at the basal end of the larva and gradually extends toward the apical end. Soon after this gradient appears the planula of *Phialidium* and of *Gonothyræa* attaches itself by the original apical end and the second gradient becomes the hydranth-stem axis. Attachment of the planula of *Stomotoca* was not observed.

5. The origin of this second polarity, opposite in direction to the first, is interpreted as a result of physiological isolation of the basal end of the planula in consequence of increase in length. From this viewpoint the hydranth-stem axis represents a process of budding not essentially different physiologically from other processes of budding in hydroids and various other forms.

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